

Blackberry Yellow Vein Associated Virus: A New Crinivirus Found in Blackberry

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Abstract

During the last three years blackberries in southern and southeastern U.S. have shown symptoms of vein clearing, yellow mottling and plant decline with considerable variation in symptoms with cultivars. We isolated dsRNA from symptomatic plants and identified high molecular weight bands similar to those isolated from plants infected with criniviruses. Paired extractions from virus-tested blackberries did not yield any dsRNA bands with molecular weight greater than 500 bp. Using degenerate primers developed against the crinivirus 1b protein in RT-PCR resulted in an amplicon that when sequenced showed the virus was a member of the *Crinivirus* genus. We have also cloned the virus and sequenced clones containing regions of the minor coat protein of the virus. Phylogenetic analysis revealed that the new virus, designated as Blackberry yellow vein associated virus (BYVaV), is related most closely to *Beet pseudo-yellow virus* and *Strawberry pallidosis associated virus*, two criniviruses recently identified in strawberry.

INTRODUCTION

Symptoms of vein clearing, yellow mottling, ringspots and plant decline have been observed in blackberry in South Carolina, North Carolina and Arkansas over the past few years (Fig. 1). Initially the symptoms were thought to be caused by *Tobacco ringspot virus* (TRSV) as this virus is known to infect blackberry and similar symptoms attributed to TRSV infection have been reported (1). Symptomatic plants were tested for 14 viruses by ELISA including: *Alfalfa mosaic*, *Arabidopsis mosaic*, *Cucumber mosaic*, *Impatiens necrotic spot*, *Prunus necrotic ringspot*, *Raspberry bushy dwarf*, *Raspberry ringspot*, *Strawberry latent ringspot*, *Strawberry mild yellow edge*, *Tobacco ringspot*, *Tobacco streak*, *Tomato ringspot*, and *Tomato spotted wilt virus* as well as for Potyviruses using group specific antibodies. However, in ELISA tests very few plants that exhibited these symptoms tested positive for TRSV or any other virus. In this report we describe a crinivirus associated with the symptoms observed in blackberry and a reverse-transcriptase polymerase chain reaction (RT-PCR) method for detecting this new virus.

MATERIALS AND METHODS

In an attempt to determine if there may be a new virus associated with these symptoms dsRNA was extracted from symptomatic and asymptomatic plants using a shortened version of the standard extraction method (2). After the dsRNA was bound to the cellulose powder in the presence of 16% ethanol in STE buffer, the samples were centrifuged for three minutes at 5,000 X g, the pellet was washed once with 50 ml of STE-EtOH and the cellulose repelleted by centrifugation. Two ml of STE was added to the final pellet and incubated at room temp for 5 min. The sample was then transferred to a microfuge tube and pellet at max rpm for 3 min. The supernatant was collected and nucleic acid precipitated by the addition of 2 ml of 95% EtOH with 5% 3 M sodium acetate. Samples were mixed then stored at -80C for 20 minutes before centrifugation in a microfuge at max speed for 30 minutes. Pellets were resuspend in 10 mM Tris-HCl containing 1 mM EDTA and analyzed by gel electrophoresis.

All enzymes were from Invitrogen Corp. (Carlsbad, CA) and used according to the manufacturer's instructions, unless noted otherwise. The dsRNA was cloned using random primers as described previously (Tzanetakis et. al, 2004). Several clones were sequenced and specific primers developed. These primers were used in RT-PCR for detection of the virus. RNA was extracted from leaf tissue using the method of Hughes and Galau (4). Phylogenetic analyses were done on the obtained sequences using the TreeTop - Phylogenetic Tree Prediction program using both cluster and topological algorithms (A.N.Belozersky Institute, Moscow State University, http://www.genebee.msu.su/services/phree_full.html).

RESULTS

DsRNA extracted from symptomatic blackberry leaves had a banding pattern in agarose gels similar to that observed for *Strawberry pallidosis associated virus* and *Beet pseudo yellows virus* (Fig. 2). Cloning with random primers followed by sequencing and searching the Genbank database using blastx database revealed sequence similarity to the minor coat protein gene (CPm) of criniviruses. Generic crinivirus polymerase primers Crini Pol F and Crini Pol R were designed and used to amplify a ~550bp fragment of the polymerase gene of the virus. Specific primers BYVV CPmF and BYVV CPmR were designed to amplify a ~180bp fragment of the CPm and primers BYVV Pol mid F and BYVV Pol mid R that amplify a ~330bp fragment of the polymerase gene of the new virus were used in RT-PCR to test for the virus in symptomatic blackberry plants from Arkansas, North Carolina and South Carolina. All plants showing symptoms tested positive for this Crinivirus, designated here as Blackberry yellow vein associated virus (BYVaV) whereas no amplification products were detected when the latter two primer pairs were used in RT-PCR tests with healthy blackberry or with strawberry infected with two other criniviruses Fig. 3). Phylogenetic analysis of 515 bp fragment from the virus polymerase (GenBank Accession number AY548174) revealed that BYVaV is most closely related to *Beet pseudo yellows virus* and *Strawberry pallidosis associated virus*, two criniviruses recently identified in strawberry that are transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*) (Fig.4).

DISCUSSION

The crinivirus detected in blackberry is associated with ringspots, yellow vein, leaf distortion ("Fig. 1) and decline in some cultivars. In 'Chickasaw' blackberry maintained in a greenhouse there were no obvious symptoms when infected with this virus. However, when these plants were taken to the field they developed dramatic foliar symptom suggesting that at least in 'Chickasaw' symptoms are caused by a synergistic reaction with an as yet undetermined virus or symptoms are dependent on environmental conditions. The symptoms described here are similar to those attributed to TRSV infection in blackberry (Stace-Smith, 1987). In this work there were several plants identified that were infected with TRSV that did not show these symptoms and other plants that showed symptoms were infected with the BYVV but not with TRSV. It is possible that this virus has been present in blackberry for many years but that the causal agent was misidentified.

The similarity of BYVaV with members of the genus *Crinivirus*, suggest a whitefly as a candidate vector as all viruses in this genus are whitefly transmitted. BPPV and SPaV have been shown to be transmitted by *T. vaporariorum* while, Lettuce infectious yellows, Cucurbit yellow stunt disorder and sweet potato chlorotic stunt are transmitted by *Bemisia tabaci*. There is a whitefly that specializes on *Rubus* species, *Aleyrodes diasemus* species, which should also be investigated as a vector.

This is the third crinivirus identified in small fruit crops during the past few years, BPPV in strawberry and blackberry (5, 6) and SPaV in strawberry (7). If these recent findings of criniviruses in small fruit crops are the result of increased whitefly populations or the naturalization of *T. vaporariorum* in parts of the USA remains to be determined. It is also possible that these viruses have not been detected in these crops previously

because of the lack of investigations. Pallidosis disease in strawberry has been recognized since the 1960's though only recently has this been shown to be a common virus in strawberry (Hokanson et al, 2000; Tzanetaksi in press). One would also expect to find BPYV in *Fragaria* and *Rubus* species in Mediterranean climates where the whitefly vector does very well since this virus and its vector *T. vaporariorum* both have very broad host ranges. We do not know how wide a host range the BYVaV has but it should be included in virus testing that is done in these areas.

Literature Cited

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Tables

Table 1. Oligonucleotide primers used for detection of Blackberry yellow vein virus.

Primer name	Nucleotide sequence 5' – 3'
Crini Pol F	GCYCCSAGRGTKAATGA
Crini Pol R	ACCTTGRGAYTTTRTCAAA
BYVV CPmF	CGTAAGAAGTTCAACATCCA
BYVV CPmR	CTTCCAGAATAGAGCACTCG
BYVV Pol mid F	CTTCAGCAGAGAGATTGGTG
BYVV Pol mid R	CCAAGAACATCGGCGAGT

Figures

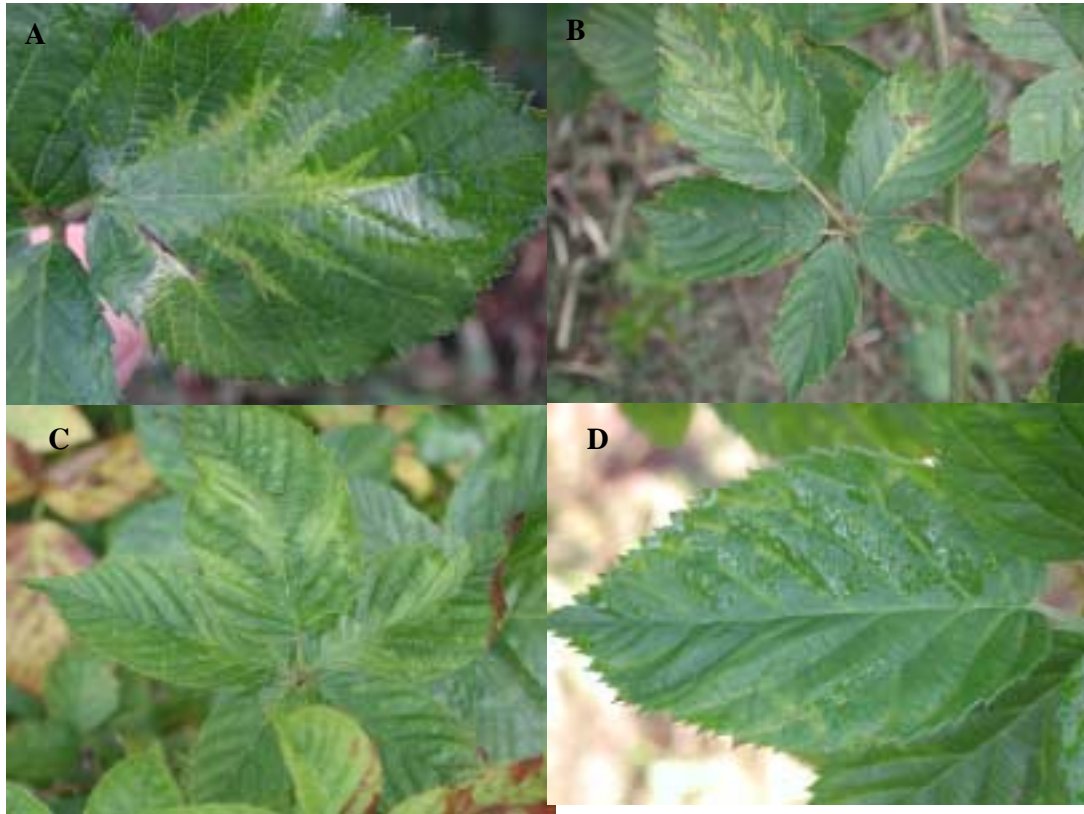


Fig. 1. Range of symptoms of Blackberry yellow vein virus on several blackberry cultivars. These images are from field plants that could have had mixed infections though they tested negative for *Raspberry bushy dwarf*, *Tobacco ringspot*, *Tomato ringspot* and *Strawberry necrotic shock* viruses. A. Arapaho; B. Navaho; C. Choctaw; and D. Kiowa.

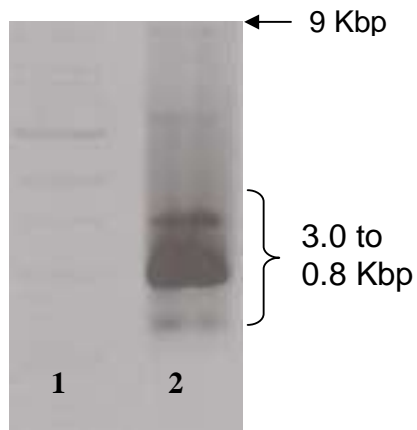


Fig. 2. dsRNA pattern obtained from blackberry infected with Blackberry yellow vein virus, lane 2; compared to dsRNA from *Beet pseudo yellows virus* infected strawberry, lane 1.

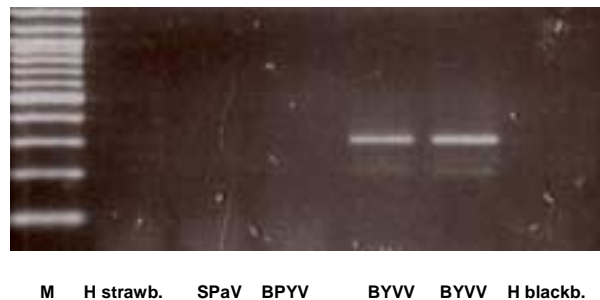


Fig. 3. Detection of Blackberry yellow vein virus from infected blackberry using RT-PCR. Ethidium bromide stained agarose gel with RT/PCR products obtained from samples: Lane 1, 100 bp ladder; Lane 2, healthy strawberry; Lane 3, *Strawberry pallidosis associated virus* infected strawberry; Lane 4, *Beet pseudo yellows virus* infected strawberry, Lane 4 and 5, Blackberry plants infected with Blackberry yellow vein virus; and Lane 6, Healthy blackberry. 330 base pair band observed in lanes 4 and 5 indicate plants were infected with the virus.

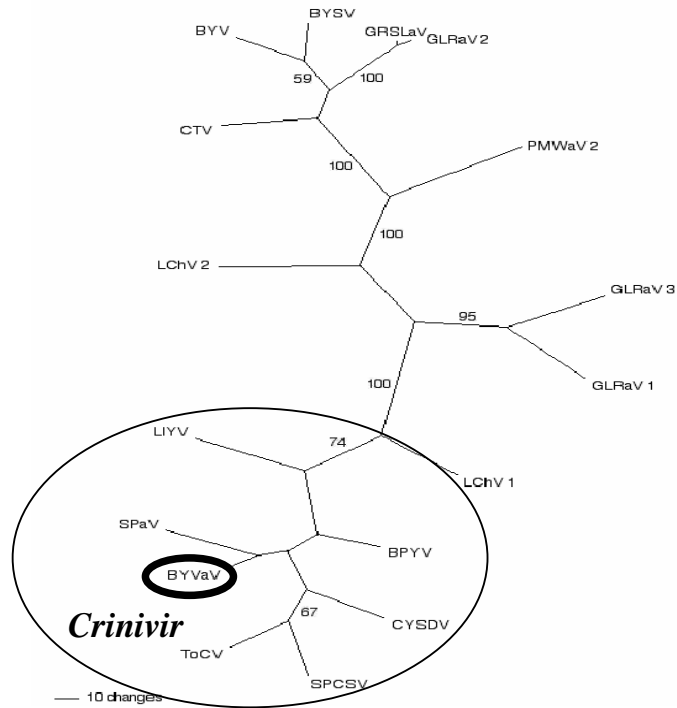


Fig. 4. Phylogenetic analysis of 172 amino acid fragment from the virus polymerase shows that BYVaV clusters with the criniviruses. Virus acronyms and GenBank Accession numbers are: *Beet pseudo yellows virus*, BPYV, NC 005209; *Strawberry pallidosis associated virus*, SPaV, AY488137; *Sweet potato chlorotic stunting virus* SPCSV, NP 689401; *Cucurbit yellow stunting disorder virus*, CYSDV, NP 851573; and *Lettuce infectious yellows virus*, LIYV, NP619695. Bootstrap values are shown as percentage value and only the nodes over 50% are labeled. The bar represents 10 amino acid changes over the length of the fragment.